CLAIMS

1. A method of determining for a given nucleic acid sample, the identity of the nucleotide at a known polymorphic site, said method comprising:

a) subjecting to an amplification regimen a population of primer extension products generated from a nucleic acid sample, each primer extension product comprising a tag sequence, which tag sequence specifically corresponds to the presence of one specific nucleotide at a known polymorphic site, wherein said amplification regimen is performed using an upstream amplification primer and a set of distinguishably labeled downstream amplification primers, each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products and a distinguishable label, wherein each distinguishable label specifically corresponds to the presence of a specific nucleotide at said polymorphic site; and

b) detecting incorporation of a distinguishable label into a nucleic acid molecule, thereby to determine the identity of the nucleotide at said polymorphic site.

2. The method of claim 1 wherein said distinguishable label is a fluorescent label.

3. The method of claim 2 wherein said step (b) comprises separating nucleic acid molecules made during said amplification regimen by size and/or by charge.

4. The method of claim 3 wherein said separating comprises capillary electrophoresis.

5. The method of claim 1 wherein said amplification regimen comprising at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers.

6. The method of claim 5 further comprising the steps, during said amplification regimen and after at least one of said reaction cycles, of removing an aliquot of said amplification reaction, separating nucleic acid molecules by size and/or by charge, and detecting the incorporation of a said distinguishable label, wherein said detecting determines the identity of the nucleotide at said polymorphic site.

7. The method of claim 6 wherein said removing, separating and detecting are performed after each cycle in said regimen.

8. The method of claim 6 wherein said separating comprises capillary electrophoresis.

9. The method of claim 1 wherein steps (a) and (b) are performed in a modular apparatus comprising a thermal cycler, a sampling device, a capillary electrophoresis device and a fluorescence detector.

10. The method of claim 1 wherein said tag sequence comprises 15 to 40 nucleotides.

11. The method of claim 1 wherein said set of distinguishably labeled downstream amplification primers consists of: a primer that comprises a tag sequence that specifically corresponds to the presence of A at the polymorphic site; a primer that comprises a tag sequence that specifically corresponds to the presence of C at the polymorphic site; a primer that comprises a tag sequence that specifically corresponds to the presence of G at the polymorphic site; and a primer that comprises a tag sequence that specifically corresponds to the presence of T at the polymorphic site.

12. The method of claim 1 wherein said set of distinguishably labeled downstream amplification primers consists of a pair of oligonucleotides, one comprising a tag sequence that specifically corresponds to a first allele of the polymorphic site and one comprising a tag sequence that specifically corresponds to a second allele of the polymorphic site.

13. The method of claim 1, further comprising the step, before step (a), of removing primers not incorporated when said population of primer extension products was made.

14. The method of claim 13 wherein said step of removing comprises degrading said primers not incorporated when said population of primer extension products was made.

15. The method of claim 14, wherein said degrading is performed using a heat labile exonuclease.

16. The method of claim 15 wherein said heat labile exonuclease is selected from the group consisting of Exonuclease I and Exonuclease VII.

17. The method of claim 16 wherein said heat labile exonuclease is thermally inactivated before continuing to step (a).

18. A method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated, said method comprising:

a) subjecting to an amplification regimen, a population of primer extension products generated from a nucleic acid sample, each primer extension product comprising a member of a set of tag sequences, which tag sequence specifically corresponds to the presence of one specific nucleotide at a known polymorphic site, wherein said amplification regimen is performed using one upstream amplification primer for each sequence comprising a known polymorphic site to be interrogated, and a set of distinguishably labeled downstream amplification primers, each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products and a distinguishable label that specifically corresponds to the presence of a specific nucleotide at said polymorphic site, and wherein said upstream amplification primers are selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product;

b) detecting incorporation of a distinguishable label in distinctly sized amplification products, thereby to determine the identity of the nucleotide at each said polymorphic site.

19. The method of claim 18 wherein said distinguishable label is a fluorescent label.

20. The method of claim 18 wherein said step (b) comprises separating nucleic acid molecules made during said amplification regimen by size and/or by charge.

21. The method of claim 20 wherein said separating comprises capillary electrophoresis.

22. The method of claim 18 wherein said amplification regimen comprising at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers.

23. The method of claim 22 further comprising the steps, during said amplification regimen and after at least one of said reaction cycles, of removing an aliquot of said amplification reaction, separating nucleic acid molecules by size and/or by charge, and detecting the incorporation of a said distinguishable label, wherein said detecting determines the identity of the nucleotide at said polymorphic site.

24. The method of claim 23 wherein said removing, separating and detecting are performed after each cycle in said regimen.

25. The method of claim 23 wherein said separating comprises capillary electrophoresis.

26. The method of claim 18 wherein steps (a) and (b) are performed in a modular apparatus comprising a thermal cycler, a sampling device, a capillary electrophoresis device and a fluorescent detector.

27. The method of claim 18 wherein said tag sequence comprises 15 to 40 nucleotides.

28. The method of claim 18 wherein said set of distinguishably labeled downstream amplification primers consists of: a subset that comprises a tag sequence that specifically corresponds to the presence of A at the polymorphic site; a subset that comprises a tag sequence that specifically corresponds to the presence of C at the polymorphic site; a subset that comprises a tag sequence that specifically corresponds to the presence of G at the polymorphic site; and a subset that comprises a tag sequence that specifically corresponds to the presence of T at the polymorphic site.

29. The method of claim 18, further comprising the step, before step (a), of removing primers not incorporated when said population of primer extension products was made.

30. The method of claim 29 wherein said step of removing comprises degrading said primers not incorporated when said population of primer extension products was made.

31. The method of claim 30, wherein said degrading is performed using a heat labile exonuclease.

32. The method of claim 31 wherein said heat labile exonuclease is selected from the group consisting of Exonuclease I and Exonuclease VII.

33. The method of claim 32 wherein said heat labile exonuclease is thermally inactivated before continuing to step (a).

34. A method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated, said method comprising:

a) subjecting to an amplification regimen, a population of primer extension products generated from a nucleic acid sample, each primer extension product comprising a first tag sequence or its complement and a member of a set of second tag sequences or its complement, the presence of which second tag sequence or its complement specifically corresponds to the presence of one specific nucleotide at a known polymorphic site, wherein for each polymorphic site in said set of polymorphic sites, said first tag sequence is located at a distinct distance 5' of said polymorphic site, relative to the distance of said first tag sequence from a polymorphic site on molecules in said sample containing other polymorphic sites, wherein said amplification regimen is performed using an upstream amplification primer comprising said first tag sequence, and a set of distinguishably labeled downstream amplification primers, each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products and a distinguishable label that specifically corresponds to the presence of a specific nucleotide at said polymorphic site, and wherein said upstream amplification primers are selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product;

b) detecting incorporation of a distinguishable label in distinctly sized amplification products, thereby to determine the identity of the nucleotide at each said polymorphic site.

35. The method of claim 34 wherein said distinguishable label is a fluorescent label.

36. The method of claim 34 wherein said step (b) comprises separating nucleic acid molecules made during said amplification regimen by size and/or by charge.

37. The method of claim 36 wherein said separating comprises capillary electrophoresis.

38. The method of claim 34 wherein said amplification regimen comprising at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers.

39. The method of claim 38 further comprising the steps, during said amplification regimen and after at least one of said reaction cycles, of removing an aliquot of said amplification reaction, separating nucleic acid molecules by size and/or by charge, and detecting the incorporation of a said distinguishable label, wherein said detecting determines the identity of the nucleotide at said polymorphic site.

40. The method of claim 39 wherein said removing, separating and detecting are performed after each cycle in said regimen.

41. The method of claim 39 wherein said separating comprises capillary electrophoresis.

42. The method of claim 34 wherein steps (a) and (b) are performed in a modular apparatus comprising a thermal cycler, a sampling device, a capillary electrophoresis device and a fluorescent detector.

43. The method of claim 34 wherein said tag sequence comprises 15 to 40 nucleotides.

44. The method of claim 34 wherein said set of distinguishably labeled downstream amplification primers consists of: a subset that comprises a tag sequence that specifically corresponds to the presence of A at the polymorphic site; a subset that comprises a tag sequence that specifically corresponds to the presence of C at the polymorphic site; a subset that comprises a tag sequence that specifically corresponds to the presence of G at the polymorphic site; and a subset that comprises a tag sequence that specifically corresponds to the presence of T at the polymorphic site.

45. The method of claim 34, further comprising the step, before step (a), of removing primers not incorporated when said population of primer extension products was made.

46. The method of claim 45 wherein said step of removing comprises degrading said primers not incorporated when said population of primer extension products was made.

47. The method of claim 46, wherein said degrading is performed using a heat labile exonuclease.

48. The method of claim 47 wherein said heat labile exonuclease is selected from the group consisting of Exonuclease I and Exonuclease VII.

49. The method of claim 48 wherein said heat labile exonuclease is thermally inactivated before continuing to step (a).

50. A method of determining the identity of a single nucleotide at a known polymorphic site, said method comprising:

I) providing a nucleic acid sample comprising said polymorphic site;

II) separating the strands of said nucleic acid sample and re-annealing in the presence of:

a) a first oligonucleotide primer comprising a 3' region that hybridizes to a sequence at a known distance upstream of said known polymorphic site, said first oligonucleotide primer comprising a first sequence tag located 5' of said 3' region; and

b) a set of second oligonucleotide primers, wherein each member of said set comprises:

i) a region that hybridizes 3' of and adjacent to said polymorphic site;

ii) a variable 3' terminal nucleotide, wherein, when said member is hybridized to said known sequence, said 3' terminal nucleotide is opposite said polymorphic site, and wherein, if and only if said 3' terminal nucleotide is complementary to the nucleotide at said polymorphic site, said 3' terminal nucleotide base pairs with said nucleotide at said polymorphic site; and

iii) a tag sequence that corresponds to said varaible 3'-terminal nucleotide of (ii), said tag sequence located 5' of the region of (i) on said member;

III) contacting the annealed oligonucleotides resulting from step (II) with a nucleic acid polymerase under conditions that permit the extension of an annealed oligonucleotide such that

extension products are generated, wherein the primer extension product from the first oligonucleotide primer, when separated from its complement, can serve as a template for the synthesis of the extension product of a member of the set of second oligonucleotide primers, and vice versa;

IV) repeating strand separating and contacting steps (II) and (III) two times, such that a population of nucleic acid molecules is generated that comprises both a sequence identical to or complementary to said first oligonucleotide and a sequence identical to or complementary to one of the members of said second set of oligonucleotides;

V) contacting the population generated in step (IV) with a heat-labile exonuclease under conditions permitting the degradation of non-annealed oligonucleotide primers, such that said primers are degraded;

VI) thermally inactivating said heat-labile exonuclease;

VII) subjecting said population of nucleic acid molecules to an amplification regimen, wherein said amplification regimen is performed using an upstream amplification primer comprising the first sequence tag comprised by said first oligonucleotide primer, and a set of downstream amplification primers, each member of said set of downstream amplification primers comprising a tag comprised by a member of said set of second oligonucleotide primers and a distinguishable label; and

VIII) detecting incorporation of at least one distinguishable label, thereby determining the identity of the nucleotide at said known polymorphic site.

51. The method of claim 50 wherein said distinguishable label is a fluorescent label.

52. The method of claim 50 wherein said step (VIII) comprises separating nucleic acid molecules made during said amplification regimen by size and/or by charge.

53. The method of claim 50 wherein said separating comprises capillary electrophoresis.

54. The method of claim 50 wherein said amplification regimen comprising at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand

separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers.

55. The method of claim 54 further comprising the steps, during said amplification regimen and after at least one of said reaction cycles, of removing an aliquot of said amplification reaction, separating nucleic acid molecules by size and/or by charge, and detecting the incorporation of a said distinguishable label, wherein said detecting determines the identity of the nucleotide at said polymorphic site.

56. The method of claim 55 wherein said removing, separating and detecting are performed after each cycle in said regimen.

57. The method of claim 50 wherein steps I-VIII are performed in a modular apparatus comprising a thermal cycler, a sampling device, a capillary electrophoresis device and a fluorescent detector.

58. The method of claim 50 wherein said tag sequences each comprise 15 to 40 nucleotides.

59. The method of claim 50 wherein said 3' region that hybridizes to a sequence at a known distance upstream of said known polymorphic site comprises 10-30 nucleotides.

60. The method of claim 50 wherein said region that hybridizes 3' of and adjacent to said polymorphic site comprises 10-30 nucleotides.

61. The method of claim 50 wherein said set of downstream amplification primers consists of: a subset that comprises a tag sequence that specifically corresponds to the presence of A at the polymorphic site; a subset that comprises a tag sequence that specifically corresponds to the presence of C at the polymorphic site; a subset that comprises a tag sequence that specifically corresponds to the presence of G at the polymorphic site; and a subset that comprises a tag sequence that specifically corresponds to the presence of T at the polymorphic site.

62. A method of determining the identities of single nucleotides present at a group of known polymorphic sites, said method comprising:

I) providing a nucleic acid sample comprising said group of polymorphic sites;

II) separating the strands of said nucleic acid sample and re-annealing in the presence of:

a) a set of first oligonucleotide primers each comprising a 3' region that hybridizes to a sequence at a known distance upstream of a known polymorphic site, each member of said set of first oligonucleotide primers comprising a common sequence tag located 5' of said 3' region, and each member of said set of first oligonucleotide primers selected such that a distinctly sized amplification product is generated for each polymorphic site in said group of known polymorphic sites; and

b) a set of downstream amplification primers comprising, in 5' to 3' order:

i) a sequence tag selected from the group consisting of a tag specifically corresponding to G as the 3'-terminal nucleotide of said primer; a tag specifically corresponding to A as the 3'-terminal nucleotide of said primer; a tag specifically corresponding to T as the 3'-terminal nucleotide of said primer; and a tag specifically corresponding to C as the 3'-terminal nucleotide of said primer;

ii) a region that specifically hybridizes to a sequence adjacent to and 3' of a polymorphic site in said group of polymorphic sites, wherein said set of downstream amplification primers comprises a subset of primers comprising a region that specifically hybridizes adjacent to said polymorphic site for each polymorphic site in said group of polymorphic sites; and

iii) a 3' terminal nucleotide selected from G, A, T or C, wherein said terminal nucleotide specifically corresponds to the sequence tag described in (i) on that downstream amplification primer, and wherein when said downstream amplification primer is hybridized to said sequence adjacent to and 3' of a polymorphic site, said 3' terminal nucleotide is opposite said polymorphic site;

III) contacting the annealed oligonucleotides resulting from step (II) with a nucleic acid polymerase under conditions that permit the extension of an annealed oligonucleotide such that extension products are generated, wherein the primer extension product from the first oligonucleotide primer, when separated from its complement, can serve as a template for the

synthesis of the extension product of as member of the set of second oligonucleotide primers, and vice versa;

IV) repeating strand separating and contacting steps (II) and (III) two times, such that a reaction mixture comprising a population of nucleic acid molecules is generated that comprises both a sequence identical to or complementary to said first oligonucleotide and a sequence identical to or complementary to a member of said set of downstream amplification primers;

V) contacting the population gnerated in step (IV) with a heat-labile exonuclease under conditions permitting the degradation of non-annealed oligonucleotide primers, such that non-annealed primers are degraded;

VI) thermally inactivating said heat-labile exonuclease;

VII) subjecting said population of nucleic acid molecules to an amplification regimen, wherein said amplification regimen is performed using an upstream amplification primer comprising the common sequence tag comprised by said first oligonucleotide primer, and a set of downstream amplification primers, each member of said set of downstream amplification primers comprising a tag comprised by a member of said set of second oligonucleotide primers and a distinguishable label; and

VIII) detecting incorporation of at least one distinguishable label, thereby determining the identities of the nucleotides present at said known polymorphic sites.

63. The method of claim 62 wherein said distinguishable label is a fluorescent label.

64. The method of claim 62 wherein said step (VIII) comprises separating nucleic acid molecules made during said amplification regimen by size and/or by charge.

65. The method of claim 64 wherein said separating comprises capillary electrophoresis.

66. The method of claim 62 wherein said amplification regimen comprising at least two amplification reaction cycles, wherein each cycle comprises the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase extension of annealed primers.

67. The method of claim 66 further comprising the steps, during said amplification regimen and after at least one of said reaction cycles, of removing an aliquot of said amplification reaction, separating nucleic acid molecules by size and/or by charge, and detecting the incorporation of a said distinguishable label, wherein said detecting determines the identity of the nucleotide at said polymorphic site.

68. The method of claim 67 wherein said removing, separating and detecting are performed after each cycle in said regimen.

69. The method of claim 62 wherein steps I-VIII are performed in a modular apparatus comprising a thermal cycler, a sampling device, a capillary electrophoresis device and a fluorescent detector.

70. The method of claim 62 wherein said tag sequences each comprise 15 to 40 nucleotides.

71. The method of claim 62 wherein said 3' region that hybridizes to a sequence at a known distance upstream of said known polymorphic site comprises 10-30 nucleotides.

72. The method of claim 62 wherein said region that hybridizes 3' of and adjacent to said polymorphic site comprises 10-30 nucleotides.

73. The method of claim 62 wherein said set of distinguishably labeled downstream amplification primers consists of: a subset that comprises a tag sequence that specifically corresponds to the presence of A at the polymorphic site; a subset that comprises a tag sequence that specifically corresponds to the presence of C at the polymorphic site; a subset that comprises a tag sequence that specifically corresponds to the presence of G at the polymorphic site; and a subset that comprises a tag sequence that specifically corresponds to the presence of T at the polymorphic site.

74. A kit for the determination of the nucleotide present at a polymorphic site present on a nucleic acid sample, said kit comprising a set of upstream primers comprising:

a) a first primer comprising a 5'-tag sequence and 3' sequence sufficient to specifically hybridize at a known distance upstream of a known polymorphic site; and

b) a set of 4 downstream second primers, comprising in 5' to 3' order:

i) a sequence tag selected from the group consisting of a tag specifically corresponding to G as the 3'-terminal nucleotide of said primer; a tag specifically corresponding to A as the 3'-terminal nucleotide of said primer; a tag specifically corresponding to T as the 3'-terminal nucleotide of said primer; and a tag specifically corresponding to C as the 3'-terminal nucleotide of said primer;

ii) a region that specifically hybridizes to a sequence adjacent to and 3' of a polymorphic site in said group of polymorphic sites, wherein said set of downstream amplification primers comprises a subset of primers comprising a region that specifically hybridizes adjacent to said polymorphic site for each polymorphic site in said group of polymorphic sites; and

iii) a 3' terminal nucleotide selected from G, A, T or C, wherein said terminal nucleotide specifically corresponds to the sequence tag described in (i) on that downstream amplification primer, and wherein when said downstream amplification primer is hybridized to said sequence adjacent to and 3' of a polymorphic site, said 3' terminal nucleotide is opposite said polymorphic site.

75. The kit of claim 74, further comprising a set of 5 primers lacking sequence specific for a gene in the genome of the organism being examined for polymorphisms, said primers comprising a primer comprising the tag sequence of said first primer and a set of four distinguishably labeled primers comprising the tag sequences of said set of four downstream second primers.